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Differential responses of exogenous melatonin on growth, photosynthesis and antioxidant defence system in two *Brassica napus* L.cultivars under chromium stress

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Abstract— *Rapid industrialization throughout the world during last few decades causing high chromium resulted widespread of agricultural soil contamination. The increased chromium contents beyond permissible level in some agricultural land areas increasing widespread concern about food safety.This study was carried out for evaluation of metal toxicity damage and its possible mitigation and improved photosynthetic efficiency by melatonin treatment in canola plants exposed to four melatonin levels (0,1,5,10µM) treated with chromium stress (0,50,100µM) for two days.Chlorophyll fluorescence a transients considered one of the best tool for photosynthetic (photosystem II) efficiency analysis of two canola cultivars Ac-Excel and DGL with or without melatonin treatment against chromium stress analyzed by using OJIP test (at different time scale) chromium treated and non-treated plants. Enhanced ROS scavenging antioxidants enzymes (SOD, POD, APX,CAT) and H_2O_2, MD Aactivity photo synthetic efficiency was observed against chromium stress. DGL cultivar showed greatly affected and showed maximum reduction in performing index of photosystem II and yield for primary photochemistry as compared to chromium treated and non-treated plants as compared to Ac-Excel. Performing index primarily comprises of active number of reaction centers as per absorption, primary photochemistry yield and efficiency of electron transfer in electron transport chain activities were observed high in Ac-Excel cultivar. However exogenous application of melatonin protected the oxygen evolving complex of PSII and helped out in maintaining PSII activity. Thus OJIP fluorescence transients are quite helpful for understanding the intersystem electron transport beyond photosystem II response of canola cultivars in chromium stress.*

Findings: *Exogenous application of Melatonin can improve plant growth and development in heavy metal stress by modulation of photosynthesis in terms of enhanced photosystem II efficiency and redox potential in certain environmental stress conditions.*

***Keywords*— OJIP, Chlorophyll fluorescence, Melatonin, chromium stress, Canola.**

I. INTRODUCTION

Heavy metals are naturally occurring trace elements of soil major source of agricultural soil contamination and major threat to food safety in several parts of the world (Ifon et al., 2019). Mostly, metals are found in soils as in the form of insoluble compounds such as oxides or carbonates, metallic complexes and free metallic ions. Among all heavy metals chromium is one of the toxic heavy metal causing severe threat to food security for many developing countries including Pakistan. Excessive use of fertilizers and pesticides, leather tanning, mining, natural disasters including volcanic eruption and weathering of rocks causing high level of chromium contents in agricultural soils (Kotecha et al., 2019). It has been found that chromium affects the physiological processes of plants mainly stunted growth, chlorosis and wilting of leaves, photosynthesis and roots damage, reduction in nutrients uptake ultimately causes death (Aparicio et al., 2019). Plants facing environmental abiotic stress conditions stimulates the formation of reactive oxygen species (ROS) which harms the production of biomolecules including nucleic acids, proteins and lipids, disturbing the sugars metabolisms and mitochondrial respiration of cell.

It has been reported that plants exposed to metal toxicity induces lipid peroxidation, which consequences membrane structure damage, enzymatic and transport activities. However, plant in response activates self-protection mechanism such as cellular enzymatic antioxidants which scavenges reactive oxygen (ROS) and reduces the oxidative stress (Rajendran et al., 2019). The hyperactivity of antioxidants enzymes and their subcellular accumulation in different parts of plants against chromium, zinc, aluminum and copper have been reported in several studies (Ghori et al., 2019). Moreover, it was observed that chromium induced toxicity leads to the poor lamellar system development, fewer grana and enhanced thylakoid lumen ultimately consequences some ultrastructure changes in plant cell (Ali et al., 2013).These changes in ultrastructure of chloroplast might have some negative impact on energy transfer imbalance and photosynthesis.

Furthermore, chromium and other trace elements may cause ultrastructure abnormalities in mesophyll cells and root cells ultimately increased metal deposition in some plant parts. Several previous studies have suggested the inhibitory effect of chromium on PSII activity mostly studies have been performed on cellular membrane damaging effect, to increase knowledge about the negative effect of chromium on photosynthesis PSII activity to evaluate the toxic effect of chromium on photosynthetic apparatus (Souri et al., 2019), Chromium stress induces changes in ultrastructure of chloroplast, electron transport chain (Chen et al., 2019). At PSII chromium ions replaces the co-factor Ca^{2+} known to be very important for water-splitting, hence alters the structure and function of oxygen evolving complex. In addition to oxygen evolving complex chromium ions interacts with many essential electron acceptor proteins i.e Q_B foundin electron transport of PSII (Oves et al., 2016; Küpper et al., 2019).

Primarily melatonin is considered as an antioxidant because of its great potential to control the reactive oxygen species under a-biotic stresses including heavy metal toxicity, salinity, drought, cold and heat stress, ozone stress, chemical pollutants, herbicides and ultraviolet radiations makes it most interesting bio-stimulating molecule for agricultural crops (Kabiri, R. et al., 2018).Several studies have indicated the potential role of melatonin in alleviation of heavy metal stress, salt stress, drought stress, heat stress in many plant species (Wang, L.Y et al., 2015). Melatonin closely associated with reactive oxygen species (ROS) generation and cell signaling under certain environmental stress conditions, thus chloroplast considered the major site of melatonin production (Martinez, V et al., 2018).

Canola (*Brassica napus* L.)is well known and major source of edible oil throughout the world. Brassica species are considered as potential candidate against heavy metal stress because of its distinguishing characteristics such as heavy metal absorption, rapid growth and greater biomass (Meng et al., 2009). Canola plants have developed specific heavy metal tolerance mechanism that enables them to grow well in polluted soil. Thus, it is necessary to evaluate the *Brassica* species response or specific mechanism involved in metal tolerance. Hence, present study was carried out to analyze the *Brassica napus* tolerance against chromium stress by exogenously applied melatonin and its effects on plant growth, chlorophyll pigments, and enzymatic antioxidant system in alleviation of metal toxicity.

II. METHODOLOGY

Plant material and growth conditions present study was carried out to investigate efficiency of PSII in adverse effects of Chromium stress on the growth of *Brassica napus* L. var. AC-Excel and DGL. A pot experiment was performed in agricultural land of Bahauddin Zakariya University,

Multan, Pakistan with normal environmental conditions (30°N and 71°28E). Seeds of canola cultivars were obtained from Ayub Agriculture Research Institute (AARI) Faisalabad. River washed sand was used as a rooting medium. In experiment 120 plastic pots with diameter 28cm with 8 kg sand were used, five to seven seeds were sown in each pot. After germination of the plants thinning was carried out leaving 4 equal distant plants in each pot. After twenty days of germination, plants were treated by various levels of Cr (0, 50, 100 µM) with Hoagland nutrient solution (full strength). After 10 days of chromium treatment foliar application of melatonin with different concentration (0, 1, 5, 10µM) mentioned as MT0, MT1, MT2, MT3 were applied exogenously to the plants. Experiment was designed according to CRBD (completely randomized block design) with three Cr levels and four melatonin levels, two cultivars and five replicates for each treatment. After twenty days of treatment, plant fast chlorophyll a kinetic analysis was measured with Fluor Pen. After taking data of all parameters mentioned above, plants were harvested. Plants root and shoots were separated. Roots were washed out and plant fresh biomass (root and shoot) were measured. For dry biomass samples of plants (root and shoot) were dried in oven at 70 °C for 48 hours, then samples weight taken in grams (g) by digital electronic balance.

Chlorophyll contents:

For the estimation of chlorophyll contents of canola plants 0.2 gram leaf tissue was taken homogenized in 80% acetone in pestle and mortar. Extract after filtration was kept 10 ml volume by adding 80% acetone in falcon tubes wrapped by aluminum foil to prevent chlorophyll degradation in light. Chlorophyll contents were measured at different wave lengths 663,652,645, and 470 nm by usingspectrophotometer (U-2900/2910 Hitachi).(Arnon, 1949).

Analysis of O-J-I-P fast chlorophyll a transients

Chlorophyll fluorescence data was recorded following nomenclature by (Kodru *et al.*, 2015) and literature related to chlorophyll fluorescence available on its manufacturer website. For this fully matured third leaves of canola plants were selected, by using hand held device Fluor Pen FP 100fluorescence transients were observed by keeping plants in dark by using aluminum foil for 30 minutes.

Antioxidants enzymes activity

Fresh plants leaf tissue of 0.1 gram was homogenized in pre-chilled pistal and mortal with 1% (w/v) polyvinyl poly pyrrolidone solution with 1.2 ml of 50mM potassium phosphate buffer by maintaining pH 7.8 along with adding 1mM EDTA-$Na_2$and 0.3% Triton X-1000 solution.

APX enzyme activity was estimated by adding 1mM ascorbate solution to prepared solution, Extract was centrifuged at 13,000 rpm for 20 minutes maintaining temperature at 4°C extract was used for following enzymes activities. SOD activity was measured according to (Zhang et al., 2013) methodology. Reaction mixture of 3 ml including 13mM methionine, 75mM NBT, 2mM riboflavin, 0.1mM EDTA and 100µL enzyme extract along with 50mM sodium phosphate buffer (pH 7.8).Reaction mixture was illuminated at light intensity of 90 for about 25 min µmol/m^{-2} s^{-1}. SOD activity was observed by measuring the enzyme extract ability or activity (µmol min^{-1} g^{-1}) of photochemical reduction of NBT (about 50%) by using spectrophotometer at 560 nm.CAT activity (µmol min^{-1} g^{-1}) was observed by reduction in absorbance of reaction mixture at 240 nm by decomposition of H_2O_2in 1 ml of reaction mixture with 50mM sodium phosphate buffer (pH 7.8) in addition 10mM H_2O_2, 20 µl of enzyme extract according to (Aebi 1984).

POD activity (µmol min^{-1} g^{-1}) was observed by preparing 1 ml reaction mixture with 100mM sodium phosphate buffer (pH 6.0) 16mM guaiacol solution 5µl of 10% H_2O_2(w/v) solution by following (Rao *et al*. 1996).APX activity (µmol min^{-1} g^{-1}) was observed by reduction in absorbance at 290 nm as reduced ascorbate was oxidized in 1 ml of reaction mixture containing 50mM hepes-KOH of 7.6 pH with 0.1mM EDTA, 0.5mM ascorbate, 0.2mM $H_2O_2$and 20µL enzyme extract, reaction was started by the addition of H_2O_2(Nakano and Asada 1981).Glutathione reductase activity was observed by following the methodology of (Griffith 1980). Fresh leaf tissue 0.1 gram were homogenized in pre-chilled pistil and mortars in 1.5 ml of 5% sulfosalicylic acid and centrifuged at 12000 rom for 15 minutes then absorbance of supernatant was used at 412 nm for measurements.

Estimation of MDA and H_2O_2

H_2O_2contents were measured by 5% trichloroacetic acid solution by following (Zhou *et al.*, 2006). MDA were measured according to the method of (Hodges *et al*., 1999).

Estimation of chromium (Cr)

For the determination of chromium contents 0.1g of dried leaf samples were taken in digestion flask with 2ml of digestion mixture was mixed and kept pre-night for about 12

hours for complete digestion of leaf plant tissue in digestion mixture. After that flasks were heated on hotplate by gradually increasing the temperature from 50^0C to 200^0C. By heating, color of plant samples turned black, at this stage about 0.5ml of $HClO_3$ was added by using dropper. After this by increasing temperature plant samples become transparent. Then flasks were taken off from hotplate, cooled and diluted by adding 50ml of distil water. Then the calculation of chromium contents were performed by using atomic absorption spectrophotometer.

III. RESULTS

Chromium toxicity causes reduction ($P \leq 0.001$)in biomass (fresh and dry weight g/plant) (Fig.1) and Leaf number and quantum yield (Fig 2) of both the canola plants that of control plants. While melatonin treated plants showed improved plant growth in terms of fresh and dry biomass and leaf number in addition to quantum yield of both canola cultivars, especially at 5µM concentration melatonin treated Ac-Excel cultivar showed significant increase in plant height, leaf number and biomass as compared to DGL cultivar with and without chromium stress.

Overall chlorophyll contents were significantly affected by chromium stress ($P \leq 0.001$) including chlorophyll a, chlorophyll b, chlorophyll a/b and total chlorophyllof canola plants (Fig.3). While exogenous application of melatonin improved chlorophyll contents meanly 5 and 10µM concentration significantly increased chlorophyll contents in chromium treated and non-treated plants. Significant increase in plant height, biomass and chlorophyll contents in Ac-Excel shows more resistance as compared to DGL in chromium stress.

Whereas plants antioxidants activity was observed to be significantly higher ($P \leq 0.001$) under chromium stress as compared to control plants in both melatonin treated and non-treated canola plants. Chromium toxicity leads to enhance the total soluble proteins contents and reactive oxygen species H_2O_2 and MDA (Fig 4) consequently more production of ROS scavenging enzymes such as superoxide dismutase (SOD), Peroxidase (POD) and Catalase (CAT) to minimizechromium toxicity (Fig 5), additionally canola plants showedhigh APX (Ascorbate peroxidase) and Glutathione reductase enzyme activity (Fig 5) to scavenge/lower H_2O_2or oxidative stress due chromium stress.

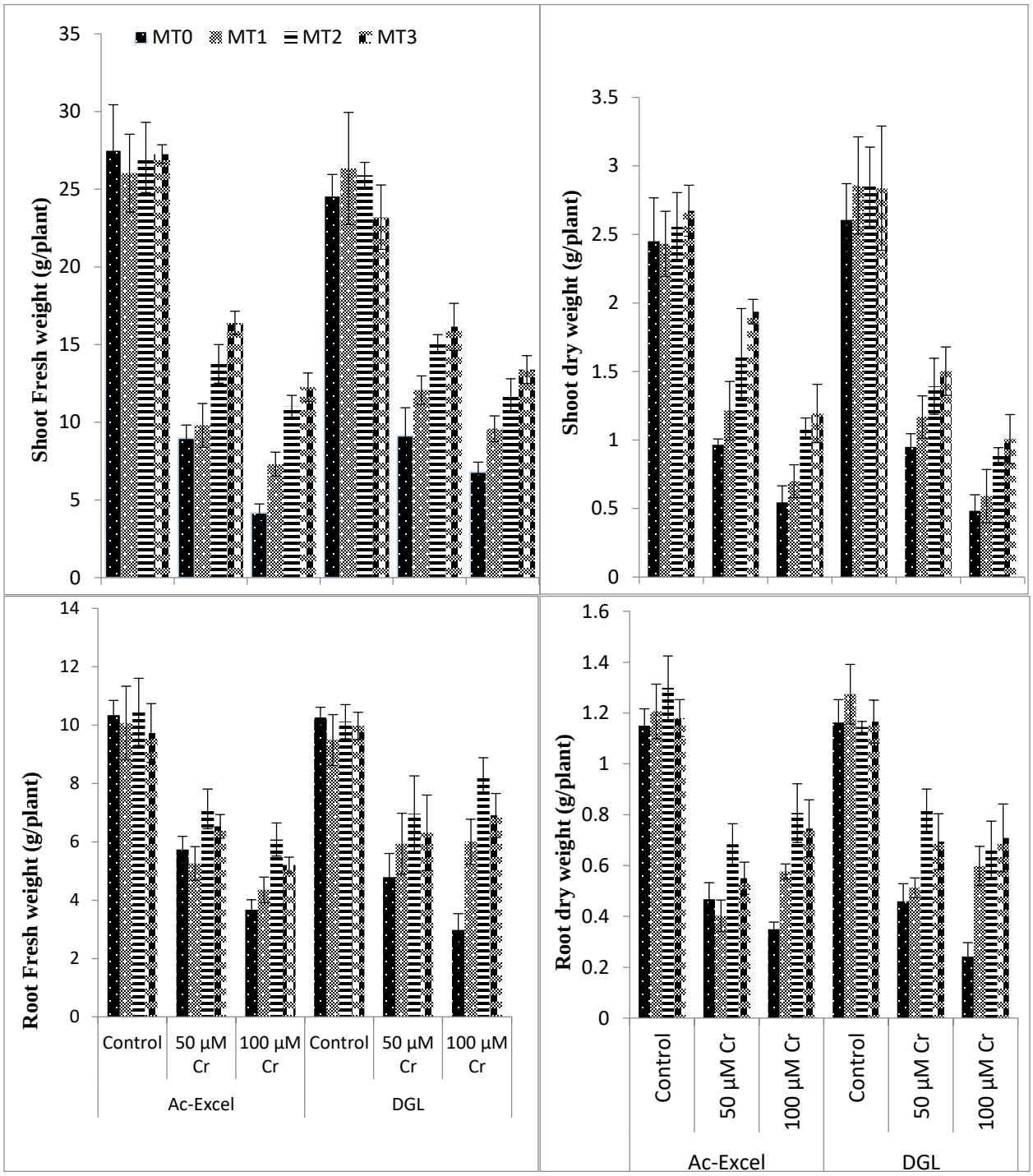

Fig.1: Melatonin induced Biomass changes of two Canola plants treated with chromium stress for two weeks.

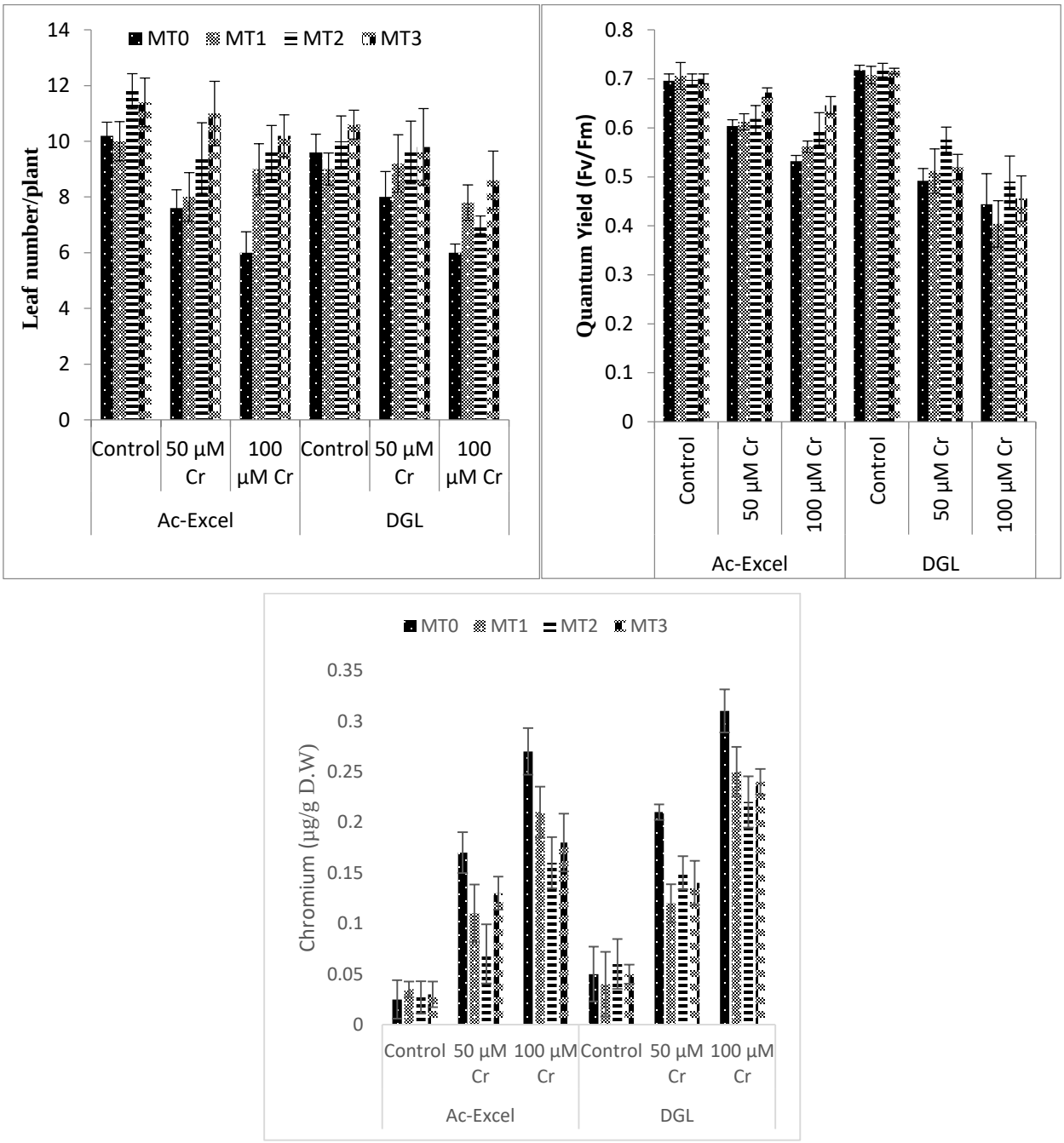

Fig.2:Melatonin induced Leaf Number/plant, Quantum yield and Chromium contents of two Canola plants treated with chromium stress for two weeks.

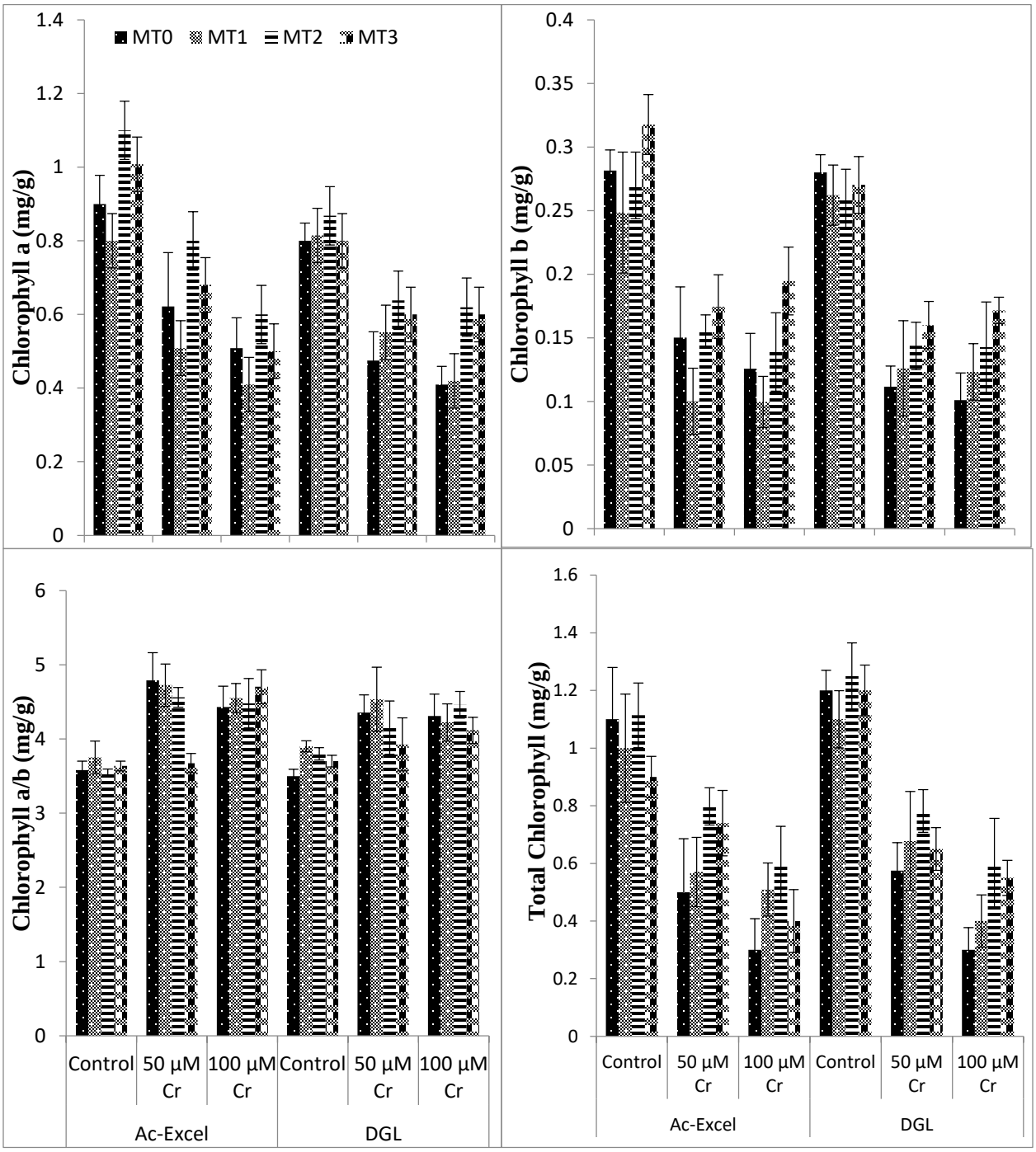

Fig.3: Melatonin induced Chlorophyll contents (mg/g) of two Canola plants treated with chromium stress for two weeks.

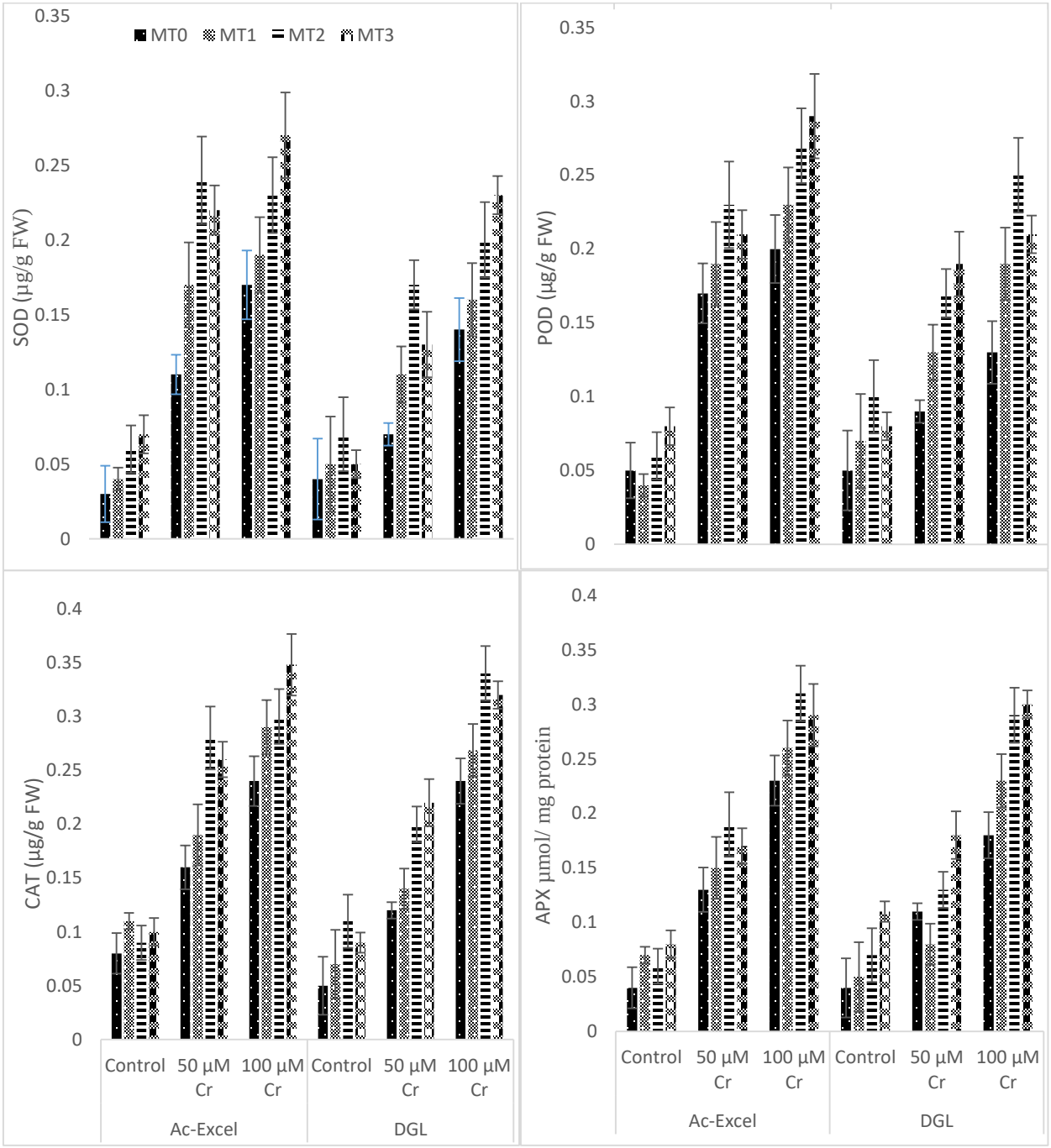

Fig.4: Melatonin induced SOD, POD, CAT, APX of two Canola plants treated with chromium stress for two weeks.

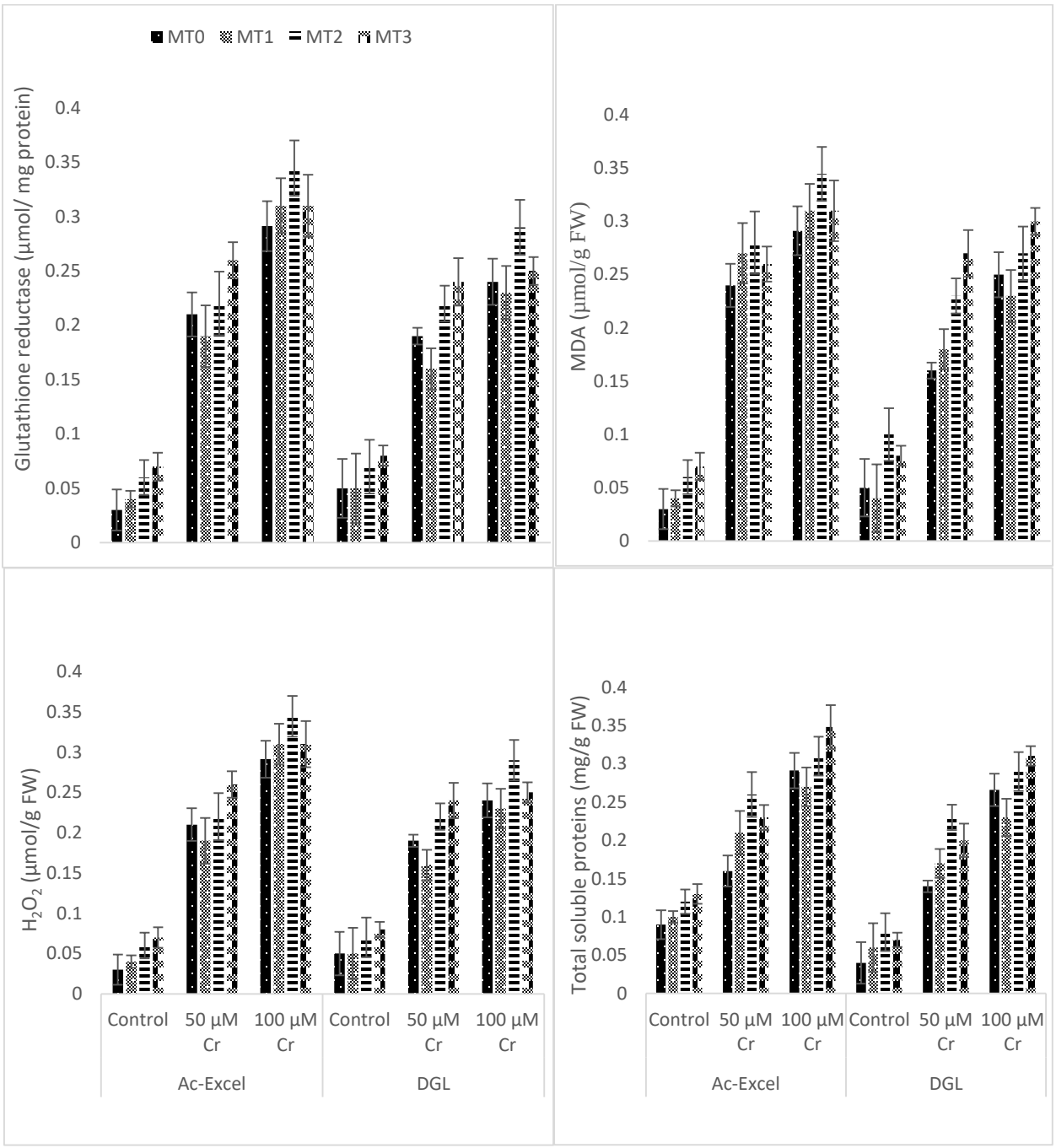

Fig.5: Melatonin induced Glutathione reductase, MDA, H_2O_2, and Total Soluble Proteins of two Canola plants treated with chromium stress for two weeks.

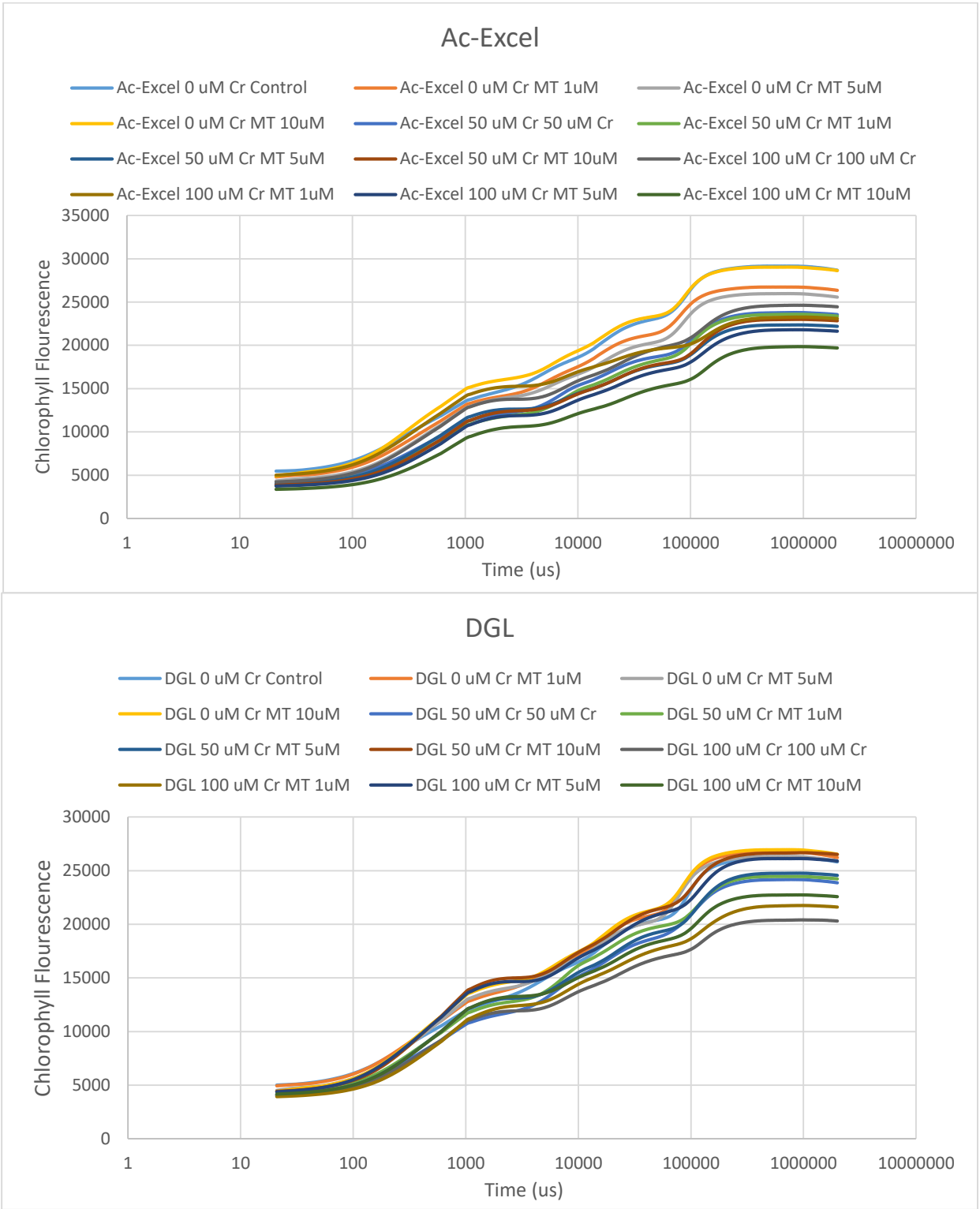

Fig.6: Melatonin induced Chlorophyll fluorescence variations of two Canola plants treated with chromium stress for two weeks.

However, melatonin treated plants showed high antioxidants enzymes activity maximum activity was observed at 5µM concentration in chromium stress as well as in control conditions. This special increase in antioxidants were higher in Ac-Excell that of DGL cultivar.

Suggesting that Ac-Excell has higher potential and tolerance of metal toxicity by showing significantly more ROS contents i.eH_2O_2 and MDA and antioxidants enzymes such as superoxide dismutase (SOD), Peroxidase (POD) and Catalase (CAT), APX (Ascorbate peroxidase) and Glutathione reductase enzyme activity.

Chromium contents were observed significantly higher in chromium treated plants in both the canola plants, but cultivar DGL showed higher chromium accumulation that of Ac-Excell cultivar suggesting that hyper accumulation of chromium shows more damaging effect on plants physiology or overall plant growth that of Ac-Excell (Fig 2).

Foliar application of melatonin increased the amplitude of I-P curve in both genotypes of canola under control conditions, while in chromium stress amplitude is lowered in both the cultivars. Suggesting that melatonin may play vital role in increasing the electron pool carrier of photosystem I end, to be reduced from electron coming from PQ in both canola cultivars. Whereas I-P band that is measured as VIP=[(Ft-FI)/(Fm-FI)] indicates that chromium stress reduces the rate of constant value of Ac-Excell melatonin enhances the rate of constant value in Ac-Excell cultivar that of DGL. Chromium stress induced biophysical changes derived from chlorophyll fluorescence curve of canola cultivars explained as in radar plot as shown in (Fig.6).

However, improved values of Fv/Fo and Fm/Fo because of melatonin treatment was observed more in Ac-Excell under chromium treated that of non-treated plants. While increased values of Mo (primary photochemistry values) in Ac-Excell cultivar was observed to be improved in foliar application of melatonin that of chromium treated plants of canola shown in (Fig.6). Similarly, total Area (PQ pool), redox state of multiple PQ turnover (Sm) and Q_A redox turnover until Fm actually (N values) was observed to be decreased in DGL only and melatonin application did not significantly affect the biophysical parameters as shown in (Fig.6). The derived fluxes of specific energy including ABS/RC (absorbance flux per reaction center), TRo/RC (trapped energy flux per reaction center), ETo/RC (electron transport flux per reaction center) and DIo/RC dissipation energy flux per reaction center all of these fully reduced in chromium stressed plants of both cultivars and melatonin treatment induced improved OJIP transients in cultivar Ac-Excel cultivar then DGL this rapid electron transfer (reduction) rate becomes faster due to chromium toxicity, because of inactive reaction centers that of control plants.

In this regard our results suggested that exogenously applied melatonin under chromium stress have higher capacity to convert light energy to chemical energy which can be used to further CO_2 to carbohydrates. Conversion of light energy into chemical energy was observed to be higher in cultivar Ac-Excell then DGL.

IV. DISCUSSION

To alleviate the chromium stress induced reduction of canola cultivars, foliar application of melatonin is considered to be one of most affective strategy (Farouk and Al-Amri, 2019) and it has been confirmed in this study. Considerably, exogenously applied melatonin in addition to endogenously melatonin increases chromium tolerance and plants antioxidants defense capacity at significant level. We supposed that melatonin induced chromium tolerance and antioxidant defense system (ROS scavenging mechanism) by production of phyto-chelatins and compartmentalization of chromium in cell wall and vacuole plays a key role in chromium tolerance for canola plants (Roychoudhury *et al.*, 2012).Similarly in the following study melatonin induced increased SOD,POD,CAT,APX and GR activity, which might modulates plants antioxidants activity by inducing ROS scavenging activity (lowering oxidative stress) against chromium stress (Fig 4,5).As melatonin treatment can decrease chlorophyll degradation, increased photosynthesis, antioxidants ability and drought tolerance cucumber seedlings (Zhang *et al.*, 2013). It is assumed that melatonin induced photosynthetic ability in plants is because of some unusual bio-stimulating pathway by modulating photosystem II efficiency in certain light and dark conditions (Zhao *et al.*, 2019).

Inside plants metal toxicity can be reduced by their reactions with metal ligands such as proteins, polysaccharides and organic acids (Andresen *et al.*, 2018) until ratio of non-chelated metallic ions changed into metabolic organelles such as nucleus, chloroplast and mitochondria. These freely available metallic ions causes severe damage to these cellular organelles. Several previous studies suggested that metallic ions sequestration in root

cortex and endodermis occurs because of decrease in transportation of metallic ions (acts as ultimately effective barrier) from root to shoot of plants (Song *et al.*, 2017). In our study exogenously applied melatonin treated plants showed decreased transportation of chromium contents in cell wall and vacuole consequently reducing the chromium toxicity suggesting that melatonin acts as barrier by reducing transportation of chromium in 50 and 100µMchromium treated canola plants. Metallic ions (chromium ions) immobilization assumed to be co-related by melatonin induced biosynthesis of thiol compounds.

Metallic ions competes with mineral nutrients for the same transport system from root to shoot resulting ionic imbalance and disturbed plasma membrane stability (Nazar *et al.*, 2012). H^+-ATPase of plasma membrane that are responsible for the translocation of organic compound and ions across the plasma membrane (Gévaudant *et al.*, 2007).Possibly melatonin improves this transportation of H^+-ATPase by its conversion into 5-methoxytryptamine that stimulates H^+-ATPase activity in addition protects plasma membrane by reducing reactive oxygen species generation and enhancing antioxidants enzymes activity(Jiang & Zhang, 2003).

Accordingly, in our study improved membrane stability, ions transportation and chromium tolerance in melatonin treated canola plants might be due to improved H^+-ATPase activity in chromium treated plants.

Chromium toxicity affects plant photosynthesis process at very large scale, whereas Fv/Fm usually acts as key indicator of plant photosynthesis ability of plant. Generally Fv/Fm always verified as a result of different pigment concentration and cell structure and can be affected by several environmental factors i.e light, nutrients, temperature and certain chemicals that alters the PSII efficiency (Li et al., 2019). Several studies explained that chromium toxicity alters the structure and function of reaction centers and effects electron transport system which consequently reduces the Fv/Fm. Furthermore our results suggested that chromium toxicity significantly alters the PSII efficiency. Melatonin application in chromium stress prevents pigment degradation that helps in improving the overall photosynthetic process. Plants exposed to metals in root region causes inhibition of growth by producing reactive oxygen species that ultimately leads to plants death (Mizushima et al., 2019).

In such conditions plant increases the endogenous melatonin biosynthesis to cope up the metal toxicity as pea plants alleviates the copper stress (Ren et al., 2019). Several studies focused on the phytoremediation ability of plants by exogenous application of melatonin exposed to metal stress by enhancing root growth, antioxidant activity, photosynthesis, by organic acid anion exudation, by reducing metal contents, by increasing antioxidants related gene expression (Arnao and Hernández-Ruiz, 2019; Zhang et al., 2019) and by reactivating the micro RNA mediated redox homoeostasis in different crops (Wang et al., 2019). Similarly, exogenous application of melatonin with 150 µmol/L for eggplant was considered as best concentration for plant against cadmium stress. Melatonin enables plants in cadmium sequestration and transformation from cytosol to vacuole and cell wall (Lv et al., 2019). In addition melatonin application mitigates heavy metal stimulated oxidative stress by enhancing enzymatic and non-enzymatic antioxidant activity (Kaya et al., 2019). Whereas melatonin has amazing efficiently to up regulate the ion channel expression against cadmium stress. However, melatonin with 1µmol concentration treatment alleviates the boron toxicity by improving nutrients uptake efficiency, photosynthetic activity, carbohydrates accumulation, antioxidant defense system and reduces reactive oxygen species (ROS) and membrane permeability in winter wheat (Qiao et al., 2019).

However, melatonin induced improvement in photosynthetic efficiency of Ac-Excel cultivar that of DGL is because of its genetic potential but its effect on the exact site of photosynthetic apparatus is still unclear. In a semi-quantitative observation of melatonin treatment with and without chromium stress on different parts of photosynthetic apparatus of canola cultivars, whole OJIP normalized transients of chromium stressed and non-stressed plants were measured. All the transient data of canola plants of present study explained that primary photochemistry fluorescence and photo electrochemical quenching at O-J and J-I step reduced due chromium stress in both canola cultivars whereas melatonin application increased the photosynthetic activity by compensating reduction rate at PSI and electron acceptor at step I-P site in Canola cultivars especially in Ac-Excel than DGL. In addition Fo normalized transient and relative variable fluorescence transients of Fo and Fm verified our results (Fig.6), for detailed analysis whole difference of kinetics at each step from OJ-JI-IP was performed (Fig.3, 4, 5). Low fluorescence values in L-band in chromium stress conditions showed that loss of energetic connectivity to some extent due to chromium toxicity.

Similarly, K-band showed both canola cultivars showed maximum ability of resistance for donor and acceptor sides of PSII imbalance at 1000 µs against chromium stress. While, an increase in K-band peaks from 1000-2000 µs showed reduced oxygen evolving complex (OEC) performance because of electron flow imbalance from (OEC) to reaction center at acceptor site of PSII in chromium stress. However, at 1000-2000 µs decreased fluorescence curve at K-band under melatonin treatment against chromium stress (Fig.4, 5) suggested that both the canola cultivars showed maximum resistance to electron imbalance at donor and acceptor sides of PSII. Similarly, at O-I step (describes redox properties of PQ poll) decreased/negative fluorescence (ΔVOI) curve described the involvement of melatonin in maintaining the maximum PQ reduction rate in both cultivars against chromium stress.

Meanwhile, fluorescence curve at I-P step that indicates the electron transfer rate from $PQH_{2\ to}$ electron accepter end of PSI, melatonin treated plants showed positive increased in fluorescence transient values at I-P step in chromium stress, while decreased in chromium treated plants suggesting that exogenous melatonin application enhanced PQ redox rate ultimately lowering the chromium stress and increased PQ pool size in both the canola cultivars. Decreased fluorescence transient curve at I-P phase eventually happens because of sharp decline of leaf water status in chromium stress that might reaches to maximum tolerance level of pants. Chlorophyll fluorescence transients and their different ratios at each step of OJIP considered as key indicators for PSII efficiency evaluation.

Chromium stress significantly reduces Fo (minimum fluorescence level) that eventually increases energy excitation and transfer rate from antenna complex to reaction center ultimately leads to low Fo. However, melatonin treated plants also have reduced Fo values, resulting increase in electron transfer efficiency from antenna complex to PSII reaction center. While Fm (maximal fluorescence) values were also reduced in chromium stress that explains the reduction in electron transfer to PSII acceptor site, indicating induced changes in Q_A reduction rate. Foliar application of melatonin enables the plants to maintain balance of plastoquinoneredox state by transferring electron to PSI. Melatonin treated plants showed reduction in ABS/RC values suggested, increased size of antenna complex of active reaction centers. However, PI most sensitive parameter of OJIP indicates the conformational changes and confirms the vitality canola plants of PSII. While exogenously applied melatonin in chromium stress increased the PI values and possible link between ETo/RC and log PI_{ABS} that suggests the utilization of PAR which reduces the CO_2 into sugars in natural environmental conditions.

V. CONCLUSION

The melatonin induced difference observed between two canola cultivars suggests that cultivars have different metal tolerance capability in chromium stress. Chromium toxicity reduced the plant growth, chlorophyll contents and photosynthetic activity in both the cultivars but DGL showed greater effect indicating more sensitive as compared to Ac-Excel. Similarly, enzymatic antioxidant activities were increased in Ac-Excell cultivar suggesting the greater metal tolerance and photosynthetic response against chromium stress. To overcome these stressful conditions exogenous application of hormones as melatonin used in our study effectively can increase the plant growth, development and tolerance against certain environmental stress. There is need to focus on exogenous application of growth enhancing agents that enables plants especially agricultural crops to increase their yield and tolerance against toxic elements.

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